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Published
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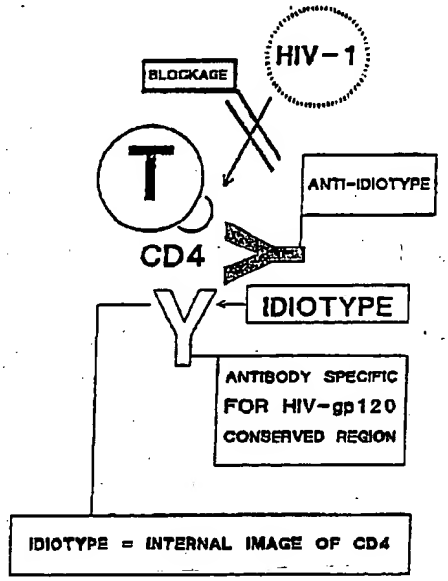
(54) Title: HIV MARKER/AIDS VACCINE

(57) Abstract

HIV infected individuals have antibodies against HIV-gp120 in their serum which have an idiotype on the antigen combining sites mimicking that of CD4 molecules. The initial anti-HIV response is accompanied by an anti-idiotypic response producing antibodies which bind to the idiotypic structure and also to CD4 molecules on T cells and other cells with this membrane marker, the HIV viral receptor. HIV is blocked by anti-idiotypic antibodies from attaching to normal CD4 cells, preventing cellular infection. Thus, a kit comprising an anti-HIV gp120 idiotype whether bound on a supporting matrix or in solution, when reacted with serum containing a gp120 anti-idiotype, will detect that antibody using appropriate immunoassay methods which may include enzymatic, radioisotope or fluorescent label, thus offering simple alternative testing methods for detection of early HIV infection. A vaccine comprising a purified anti-HIV gp120 antibody with the specific configuration of having an idiotype which mimics a CD4 molecule is also contemplated.



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+ DESIGNATIONS OF "SU"

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HIV MARKER/AIDS VACCINE

Field of Invention

This invention relates to the fields of immunology and virology and pertains to immunochemical identification of HIV-1 infection based upon detection of anti-idiotypic antibodies in the serum of HIV-1 infected individuals, and to vaccines for the treatment of HIV.

Background of Invention

The accepted current methods for HIV antibody testing may well be inadequate to detect all people infected with HIV-1. This has also been suggested recently by other investigators using the sensitive, complex and expensive test - PCR (polymerase chain reaction). A pilot study has now identified a new sensitive marker for infection, one that could be used on both small and massive scales, e.g., to monitor volunteer blood donations by the Red Cross.

This marker is a novel serum autoantibody (serological marker referred to as anti-idiotypic antibody) which has the unique property of specifically reacting to both special determinants (called idiotypes) on the antigen combining sites of anti-HIV gp120 antibody and CD4 molecules on T lymphocytes. It is proposed that these antibodies may have a protective role against HIV infectivity and spread during the asymptomatic period of HIV infection. The mechanism by which anti-HIV anti-idiotypic antibodies might provide protection during the early "latency" period and the immunologic mechanisms leading to the loss of protection and progression of disease is currently being investigated.

Acquired immune deficiency syndrome (AIDS) is a disorder caused by infection with Human Immunodeficiency Virus (HIV). Infection with HIV in some individuals results in a profound immunological defect resulting in opportunistic infections with devastating results and eventual death of the patient. In other individuals, HIV infection may produce no pathological complications or may result in mild to moderate abnormalities, collectively termed AIDS Related Complex (ARC). The factors responsible for the progression from ARC tc AIDS are not known. Furthermore, it is also not known whether all individuals who are assymptomatic seropositives eventually progress to AIDS and while most do, some do not. The elements that control these dynamics remain to be defined.

The mechanism of this profound immune deficiency is also not completely clear. Although there is considerable evidence to suggest that infection of $CD4^+$ (helper/inducer) T lymphocytes by HIV results in the death of these cells, this may or may not be an important cause of immune deficiency. The molecular mechanisms involved in this cell death are also not clear, although direct cytotopathic effect, cell fusion and giant cell formation resulting in cell death and lymphotoxin production have all been postulated. All these mechanisms, however, fall short of explaining a number of observations which are contrary to cell death as the only mechanism of immune deficiency in this disease. For example,

it has been observed that in patients who are positive for HIV, only 1:100 to 1:100,000 $CD4^+$ T cells are infected with the virus. Moreover, immunoglobulin levels in AIDS patients are often higher than controls. This paradoxical observation has been explained on the basis of a hypothetical polyclonal stimulation of B cells (similar to Pokeweed mitogen) by HIV. However, HIV antigens, on the contrary, have been shown to inhibit mitogen responses of peripheral blood lymphocytes and T cells from AIDS patients respond poorly to mitogens. Recently, it has been shown that the FcRIII receptor on human macrophages and possibly another Fc receptor on human CD4 lymphocytes can mediate antibody-dependent enhancement of HIV infectivity and that this phenomenon proceeds through a mechanism independent of the CD4 receptor.

The antibody response of HIV infected patients to HIV antigens is well known. It appears that early in the infection patients make antibodies to both the core proteins (gag gene to make Pr55 and enzymatic cleavage to p17, p24 and α $p15^*$) and the envelope proteins (env gene to make gp160 and enzymatic cleavage to gp120 and gp41) of the virus. However, with the progression of the disease the antibodies to core proteins are diminished or disappear while the antibodies to envelope proteins remain elevated. It has also been shown that a large percentage of AIDS patients have circulating immune complexes in their sera suggestive of an infectious etiology since isolated immune complex material from such

patients has significant quantities of HIV antigen or antibody. Recent reports have indicated the presence of autoantibodies reactive against cardiolipin and HIV-1 accessary gene products such as viral negative factor (nef), rev, tat, vpu and vpr, lymphocytes, etc. Taken together, all these observations suggest that other mechanisms of immunosuppression may in fact be operative in these patients and may indeed contribute significantly in the induction and maintenance of immunosuppression in AIDS.

The high frequency and high titre of anti-HIV gp120 antibodies in the serum of infected subjects would be expected to provide protection from progression to AIDS. On the contrary, infected individuals can progress to AIDS, even in the presence of antibodies to HIV-gp120. Thus, antibodies as such appear to have little or no correlation with immune protection against the development of AIDS. The significance of these antibodies in HIV infected subjects remains elusive. One such mechanism that has not yet been studied in these patients is the down-regulation of the immune response by auto-anti-idiotype antibodies, although this has recently been postulated as a mechanism of immunosuppression in AIDS. An AIDS-related cytotoxic auto-antibody that reacts with $CD4^+$ T cells has been shown to be present in the serum of patients with AIDS and ARC.

In AIDS, it is clear now that the CD4 molecule is important in HIV attachment, penetration and eventual death

of helper T cells and, in fact, is the viral receptor per se. Moreover, there have been several demonstrations of anti-lymphocyte antibodies in the serum of individuals infected with HIV. In most cases, reactivity did not seem to be directed towards a specific lymphocyte population. However, others have demonstrated that these antibodies did not bind at random but reacted predominately with CD2+/CD4+ cells. Some studies indicate that the CD4 molecule is masked by circulating antibodies in AIDS patients. The genesis of such antibodies and their contribution to the pathogenesis of the immune deficit during HIV infection have not been fully investigated. A statistical link has recently been demonstrated between the presence of anti-lymphocyte antibodies (i.e. anti- CD4+ T cells) and the progression to AIDS. 176/200 (88%) AIDS patients and 4/50 (8%) HIV-seropositive non-AIDS patients had significant levels of anti-CD4+ antibodies. In patients with ARC, 64% were found to be positive for anti- CD4+ antibodies, and in 59% of these, the disease progressed to AIDS within 30 months. Interestingly, the 36% who had no significant levels of antibodies had no progression. Recent studies have indicated that 10% of HIV-1 infected individuals produce antibodies that recognize the extracellular portion of the CD4 molecule, a region that is distinct from the virus-binding domain. These studies fail to demonstrate the origin and characteristics of these anti-lymphocyte (CD4 specific) antibodies. Recent reports have

indicated that serum antilymphocyte antibodies could lead to the development of AIDS in HIV-seropositive men. It is noteworthy that before the onset of clinical AIDS, progressors can be distinguished from nonprogressors by markedly different rates of CD4 cell depletion and virus replication. The factors that control these processes remain to be determined. Autoantibodies have been implicated leading to immune damage in AIDS.

For a number of years, the role of anti-idiotypic antibodies in their capacity to suppress immune responses has been studied. Classically, the individual antigenic specificities of homogeneous antibodies or antigen-specific cell receptors are called idiotypes. Antibodies to such idiotypes are referred to as anti-idiotypes. One of the remarkable properties of anti-idiotypic antibodies is the molecular mimicry of biological receptors. For example, measles virus anti-idiotype inhibits virus infection of primate cells. In this system, it has been reported that (a) anti-idiotype against anti-measles virus when pre-incubated with Vero cell monolayers, inhibited infection of these cells by measles virus, and (b) anti-idiotype induced complement-mediated lysis of Vero cells. Thus, anti-idiotype in the measles system has the potential for binding to and mediating destruction of host cells bearing the measles virus receptor. In other examples, anti-idiotypic antibodies have been shown to react against virus receptors for retrovirus and hepatitis B on infected cells.

Evidence supporting the pathogenic potential of anti-idiotypic antibodies includes the presence of glomerular immune deposits in rabbits with serum sickness induced by chronic intravenous administration of bovine serum albumin (BSA) containing auto-antibodies. Similarly, in mice, immune deposits induced by injection of bacterial lipopoly-saccharide were found to contain idiotypic as well anti-idiotypic immunoglobulin molecules. In addition, anti-idiotypic antibodies (anti-anti-casein) have been shown to be produced in man following milk ingestion. Others have found anti-idiotypic, insulin-mimicking antibodies in the blood of some people suffering from the autoimmune form of diabetes nellitus.

These observations suggest that anti-idiotypic antibodies play an important role in AIDS. Anti-idiotypic antibodies can react to the antigenic determinant (idiotype) of the primary anti-HIV gp120 antibody and thus, even in the absence of persistent viral replication, may cross-react with receptor-bearing $CD4^+$ T lymphocytes as well as on other cells (eg. monocytes or neuronal cells). This mechanism of binding can be directed either to a non-viral binding region on the CD4 molecule or to an Fc receptor. Interestingly, HIV gp120 glycoprotein has been shown to share a cross-reacting epitope with a surface protein on activated human monocytes and is involved in antigen presentation. This in turn may potentiate the destruction of non-infected $CD4^+$ T cells which in

turn may enhance "opportunistic" infections or even neurological diseases. There is growing recognition that AIDS patients can develop neurological diseases, ranging from peripheral neuropathy to fulminant dementia, with or without a clinically evident immunodeficiency. Explanations for neurological disorders in AIDS have been proposed to be a competitive effect of HIV gp120 for neuroleukin receptors on neurons since there is some homology between gp120 and neuroleukin.

If antigenic mimicry exists between anti-HIV gp120 idiotype and CD4 molecule, then anti-idiotypic antibodies could conceivably play a protective role in disease progression.

Object of the Invention

Thus, it is one object of the present invention to provide an improved method for the detection of early HIV infection.

Another object of the present invention is to provide a diagnostic kit for the determination of early HIV infection.

Yet another object of the present invention is to provide a vaccine for treatment of HIV infection.

Brief Statement of Invention

Thus by one aspect of this invention there is provided a method for detecting anti-HIV gp120 anti-idiotypes in a biological fluid comprising: a method of detecting HIV infection in a biological fluid, comprising:

a) contacting an anti HIV gp120 idiotype with a biological fluid under conditions which allow formation of complexes between anti-HIV gp120 idiotypes and HIV gp anti-idiotypes in said biological fluid; and

b) detecting the formation of complexes as an indication of the presence of HIV gp120 anti-idiotypes in said biological fluid.

By another aspect of this invention there is provided a di gnostic kit for the detection of HIV anti-idiotypic antibodies in patient serum, comprising:

(a) a supporting matrix having immobilized thereon an anti-HIV gp120 idiotype; and

(b) a labelled antibody directed against a second site on said idiotype.

By another aspect of this invention there is provided a vaccine comprising a purified anti-HIV gp120 antibody with a specific configuration of an idiotype which mimics a CD4 molecule.

Brief Description of Drawings

Fig. 1 is a sketch illustrating a model for anti-idiotypic antibodies in AIDS;

Fig. 2 is a graph illustrating anti-idiotypic reactivity over time;

Fig. 3 is a histogram of OKT4 binding to MOLTL T cells and inhibition of binding by anti-idiotypic antibodies in patient serum;

Detailed Description of Preferred Embodiments

In preliminary work, a commercially obtained affinity purified sheep IgG polyclonal antibody (D7324, International Enzymes, CA) specific for the conserved epitope regions of HIV-1 gp120 has been tested using a synthetic analogue of gp120 called peptide T (Bachem, California). Peptide T also binds CD4. This antibody (D7324) will also have a conserved idiotype. Thus, anti-idiotypic antibodies to the conserved idiotype will not be missed in serum due to HIV antigenic variations. It has been found that the sheep anti-gp120 antibody binds to peptide T in an ELISA assay, and it can be inhibited (64%) from binding to peptide T by Mab OKT4 (Ortho). Mab anti-gp120 from Dupont as well as Mab OKT4 do not bind to peptide T. Some of the ARC patients' serum antibodies exhibited binding to peptide T and were inhibited (in the range 21-80%) by Mab OKT4. From this experiment, the data indicate that the polyclonal sheep anti-gp120 antibody as well as some of the patient's antibodies exhibit an idiotype that behaves as an internal image of CD4 (see Figure 1). In this figure, it is proposed that anti-idiotypic antibody not only binds to the idiotope of anti-HIV gp120, but also to CD4, and thus, may block HIV infectivity.

Furthermore, after biotinylation of the sheep IgG anti-gp120, the antibody was used as a probe to detect anti-idiotype antibodies in HIV-infected patient's serum after isoelectric focusing across a broad pH gradient on agarose

gels and capillary blotted onto nitrocellulose paper. After extensive washing and blocking with Tween-20, alkaline phosphatase conjugated streptavidin was added and the reaction developed for color using BCIP (5-bromo-4-chloro-3-indolyl phosphate-p-toluidine) and NBT (nitroblue tetrazolium) (Bio-Rad).

Adsorption of the serum on a protein A column removed the activity. Table 1 summarizes the results.

Table 1.

Frequency of patients positive for anti-idiotype in serum

Patient Status	Number of patients (+)		Chi-sq	p values
Normal subjects	0/12			
Autoimmune uveitis	0/1	0%		
Asymptomatics	6/9	66%	11.200	8.18×10^{-4}
ARC	15/18	83%	20.000	7.74×10^{-6}
AIDS	2/8	25%	3.333	n.s.
AIDS + PCP	2/8	25%	3.333	n.s.
AIDS + Kaposi's	3/3	100%	15.000	1.08×10^{-4}
AIDS + Wasting	2/2	100%	14.000	1.83×10^{-4}
SERONEGATIVE COHORT	13/13	100%	25.000	5.73×10^{-7}

It is critical to note that there are a large number of patients with ARC, seropositive asymptomatics, and particularly high risk seronegative gay cohort males with detectable anti-idiotypic antibodies in their serum. In addition, patients with Kaposi's and wasting syndrome had detectable

anti-idiotypic antibodies. In contrast, only a few patients with AIDS and PCP had detectable anti-idiotypic antibody. Our control subjects, including normal, healthy individuals (0/12), a patient with autoimmune uveitis (0/1), normal subjects (data not in figure) immunized with influenza vaccine (0/4), individuals infected with measles (1/3), CMV (0/2), mumps (0/2) or VZ (0/3), have very little or no detectable amounts of serum anti-HIV anti-idiotypic antibodies.

Seronegative gay males were chosen for their known history of frequent sexual contacts with persons diagnosed as ARC or AIDS. Although the presence of strong anti-idiotypic antibody reactions suggests that they are infected with HIV, all 13 persons remain well to date after up to 5 years of follow-up. In an early study, 20/21 seronegative gay males seroconverted with a positive PCR for HIV, and the one who remained seronegative had a positive PCR test for HIV. In a three month follow-up study, 3 of 3 patients showed marked increases in their anti-idiotypic reactivity (Figure 2). In a recent report, others have shown that a select seronegative cohort group at high risk for HIV infection had circulating B cells that, upon in vitro polyclonal activation with pokeweed mitogen, produced anti-HIV antibodies which correlated well with PCR analysis of DNA of their cells for HIV-specific sequences.

Furthermore, adsorption of the serum on a protein A column which has an affinity for IgG antibodies, essentially removes the activity, suggesting that the anti-idiotypic

antibodies are of the IgG isotype. In another study, patient's serum previously determined to have strong anti-idiotypic antibodies reactivity was selected, and its binding to CD4 molecules on MOLT-4 cell lines determined. In this experiment, a blocking assay was used in which serum antibodies would block FITC conjugated Mab OKT4 from binding to CD4 on MOLT-4 T cells, using flow cytometry. As shown in Figure 3, a typical histogram of OKT4 binding to MOLT-4 cells and inhibition of binding by serum #77 (1/100 dilution) containing anti-HIV anti-idiotypic antibodies. The same serum #77 inhibited Mab OKT4 staining of MOLT-4 cells by 38% at 1/10 dilution, by 27% at 1/1000 dilution.

From the above, it is clear that a diagnostic method and apparatus for the detection of HIV infection can be developed.

The method and test kit is a qualitative as well as a quantitative, non-instrumental, test strip enzyme immunoassay for anti-HIV gp120 idiotypic antibody in patient serum, which is based on the activity of a tracer. The total HIV anti-idiotypic antibody enzyme immuno-assay kit is a two sites method using two antibodies, monoclonal or polyclonal, directed against two different parts of the protein in question. The first antibody, or idiotype thereof, may be fixed on a supporting matrix, such as a nitrocellulose or nylon disk, preferably mounted for ease of handling at one end of a plastic strip, the second antibody is labelled with an enzyme, such as an alkaline phosphatase.

There are three essential components for the enzymiomtic assay: (a) a supporting matrix, such as a dry nitrocellulose or nylon disk, preferably, but not essentially, attached to a plastic strip for ease of handling, to which has been immobilized a specific monoclonal or polyclonal antibody, or idiotype thereof against the antibody of interest, namely an HIV gp120 idiotypic antibody such as a sheep IgG polyclonal antibody (D7324, International Enzymes, CA) specific for the conserved epitope regions of HIV-1 gp120; (b) an enzyme reagent containing an alkaline phosphatase conjugate of a second monoclonal or polyclonal antibody directed against a different site on the protein of interest, such as alkaline phosphatase conjugated goat anti-IgG and (c) a colour developer solution containing substrate for the enzyme, such as BCIP (5-bromo-4-chloro-3-indolyl phosphate-p-toluidine) and NBT (nitroblue tetrazolium) (supplied by Bio-Rad, CA).

It will be appreciated, however, that other forms of immuno-assay kits are also contemplated by this invention. For example, the labelled antibody may equally well be labelled with a radioisotope or a fluorescent material.

Example

Patient serum samples were transferred to a container, and an antibody test strip comprising D7324 (sheep IgG polyclonal antibody) on a dry nitrocellulose disk was inserted into the samples and incubated at ambient temperature for 30

minutes with agitation. The strip was then removed and washed extensively in a buffer solution. The strip was then immersed in a tracer solution containing Tween-20^{R}, and an alkaline phosphatase conjugated goat anti-IgG and incubated for 1 hour with agitation (overnight without agitation). The strip was then washed in buffer solution and dipped into a colour developer solution containing 5-bromo-4-chloro-3-indolyl phosphate-p-toluidine and nitroblue tetrazolium. The disk showed an insoluble blue product resulting from coupled enzyme reactions involving the enzyme, alkaline phosphatase and its substrate. The colour intensity was related quantitively to the amount of the anti-HIV gp120 antibody in the patient serum and it was possible to construct a result table of known amounts of protein as well as negative controls.

As previously noted, mimicry between anti-HIV gp120 idiotype and the CD4 molecule provides a means of protection against HIV progression. Thus a vaccine comprising a purified anti-HIV gp120 antibody with a specific configuration which mimics the CD4 molecule should have therapeutic value in inhibiting progression of the HIV positive patient to full blown AIDS.

We Claim:

1. A method of detecting HIV infection in a biological fluid, comprising:

a) contacting an anti-HIV gp120 idiotype with a biological fluid under conditions which allow formation of complexes between anti-HIV gp120 idiotypes and HIV gp anti-idiotypes in said biological fluid; and

b) detecting the formation of complexes as an indication of the presence of HIV gp120 anti-idiotypes in said biological fluid.

2. A method as claimed in claim 1 characterized by providing a supporting matrix on which is immobilized anti-HIV gp120 idiotype; incubating said supporting matrix with a sample of said biological fluid; separating said supporting matrix from the sample and determining the antibody bound to the supporting matrix as an indication of the presence of HIV gp120 anti-idiotype in the biological fluid.

3. A method as claimed in claim 2 characterized in that the step of determining the antibody bound to the supporting matrix comprises: incubating the supporting matrix with a labelled antibody which is directed against a second site on the HIV gp120 anti-idiotype; separating the supporting matrix from the labelled antibody; and detecting the label associated with the supporting matrix as an indication of HIV gp120 anti-idiotype in the biological fluid.

4. A method as claimed in claim 3 characterized in that said label is selected from the group consisting of an enzyme, a radioisotope and a fluorescent material.

5. A method as claimed in claim 4 characterized in that said label is an enzyme reagent conjugated to said antibody directed against a second site on said idiotype, and further characterized by immersing said enzyme treated matrix in a colour developer solution containing a substrate for said enzyme to thereby produce an insoluble colour deposit on said matrix indicative of the presence of HIV gp120 idiotypes in said biological fluid.

6. A method as claimed in any of claims 1-5 characterized in that biological fluid is selected from human serum and human plasma.

7. A method as claimed in any of claims 1-6 characterized in that said anti-HIV gp120 idiotype is directed against a conserved epitope of gp120.

8. A method as claimed in any of claims 1-7 characterized in that said labelled antibody is labelled anti-human IgG antibody.

9. A method as claimed in claim 5 characterized in that said anti-HIV gp120 idiotype is a sheep IgG polyclonal antibody specific for a conserved epitope region of HIV-1 gp120.

10. A method as claimed in claim 9 characterized in that said solution containing an enzyme reagent comprises alkaline phosphatase conjugated goat anti-IgG.

11. A method as claimed in claim 9 characterized in that said enzyme reagent comprises alkaline phosphatase conjugated streptavidin.

12. A method as claimed in claim 10 characterized in that said colour developer solution comprises 5-bromo-4-chloro-3-indolyl phosphate-p-toluidine and nitroblue tetrazolium.

13. A kit for detecting HIV gp120 anti-idiotype in a biological fluid, comprising, in separate containers, the components:

(a) a supporting matrix on which is immobilized anti-HIV gp120 idiotype; and

(b) a labelled antibody directed against a second site on the anti-idiotype.

14. A kit as claimed in claim 13 characterized in that said labelled antibody is selected from the group consisting of an enzyme conjugated antibody, a radioisotope labelled antibody and a fluorescent labelled antibody.

15. A kit as claimed in claim 14 wherein said labelled antibody is an enzyme conjugated antibody, and further characterized in that said kit includes a colour developer solution containing substrate for said enzyme.

16. A kit as claimed in claim 14 or 15 characterized in that said immobilized anti-HIV gp120 idiotype is a sheep IgG polyclonal antibody specific for conserved epitope regions of HIV-1 gp120.

17. A composition for treatment of HIV infection comprising HIV gp120 anti-idiotype, in an amount effective to block HIV infection of CD4 receptor-bearing cells, in a physiologically acceptable carrier.

18. A vaccine comprising a purified anti-HIV gp120 antibody with a specific configuration of having an idiotype which mimics a CD4 molecule.

19. A vaccine as claimed in claim 18 characterized in that said antibody is an affinity purified antibody.

20. A vaccine as claimed in claim 18 characterized in that said antibody is a monoclonal antibody.

21. A method of treating HIV infection comprising administering the vaccine of claims 18, 19 or 20 to an HIV infected subject.

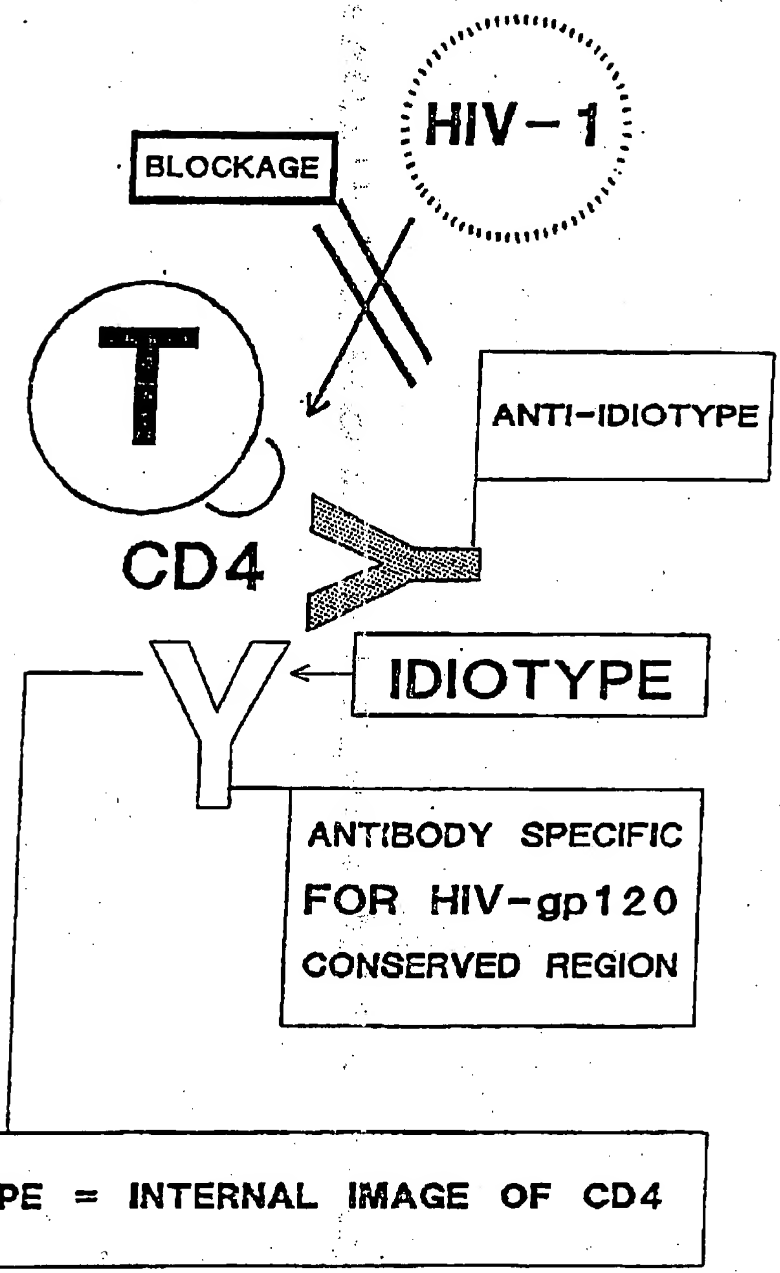

FIGURE 1

SUBSTITUTE SHEET

SERONEGATIVES: FREQUENT SEXUAL CONTACTS WITH HIV INFECTED PATNERS

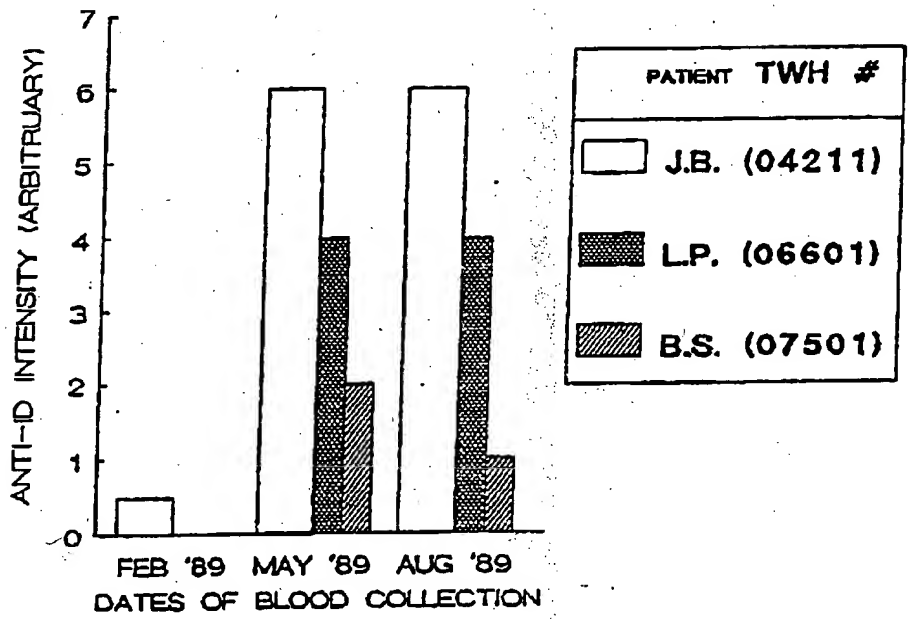

FIGURE 2

SUBSTITUTE SHEET

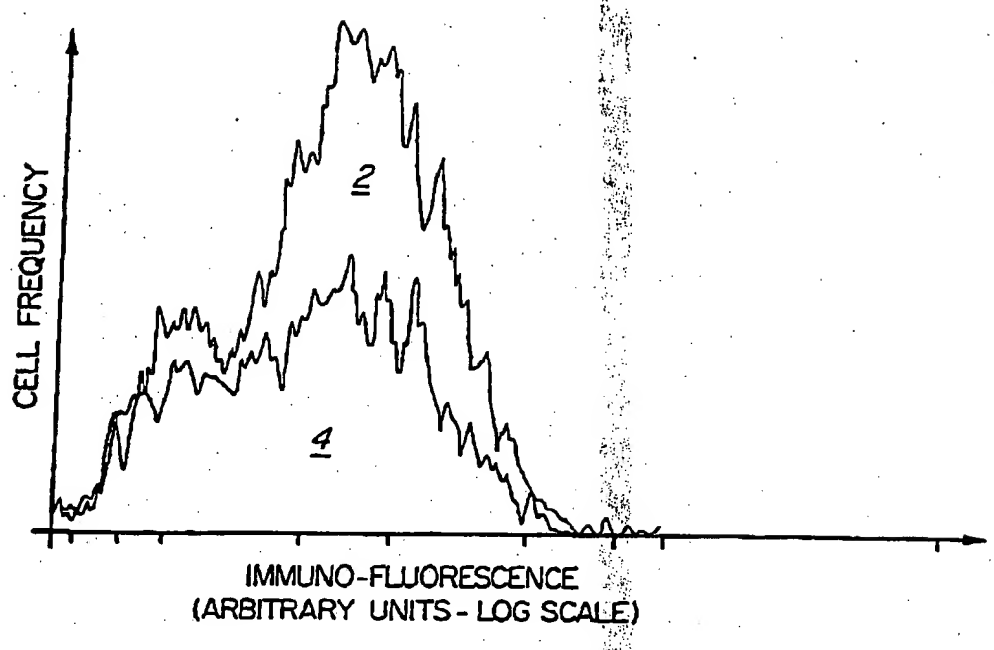

FIGURE 3

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International Application No PCT/CA 91/00404

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 G01N33/569; G01N33/68; C07K15/00; C12P21/00
A61K39/395

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	G01N ; C07K ; A61K ; C12P

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[10]	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
Y	THE JOURNAL OF IMMUNOLOGY vol. 145, no. 7, 1 October 1990, BALTIMORE MD USA pages 2199 - 2206; M.S.C. FUNG ET AL: 'MONOCLONAL ANTI-IDIOTYPIC ANTIBODY MIMICKING THE PRINCIPAL NEUTRALISATION SITE IN HIV - 1 GP 120 INDUCES HIV - 1 NEUTRALIZING ANTIBODIES IN RABBITS.' See whole article. ---	1-16
Y	R.A. MORISSET. 'V International conference on aids: the scientific and social challenge.' 1989 , INTERNATIONAL DEVELOPMENT RESEARCH CENTRE , OTTAWA ONTARIO CANADA See page 650 abstract nr. c.553 : J.J. Drabick et al : "Naturally occurring auto-antibodies to CD4 and the MHC class II molecule in HIV - infected patients". --- -/--	1-16

[10] Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
17 FEBRUARY 1992	02 MAR 1992
International Searching Authority	**Signature of Authorized Officer**
EUR PEAN PATENT OFFICE	VAN BOHEMEN C.G.

Form PCT/ISA/210 (second sheet) (January 1985)

International Application No PCT/CA 91/00404

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	WO,A,8 809 181 (TANOX BIOSYSTEMS) 1 December 1988 see page 16, line 6 - line 10 see page 95, line 6 - line 23	18-21
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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. CA 9100404 SA 53004

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
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